Appendix C

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Mini review

Extended longevity and insulin signaling in adipose tissue

Nora Klöting[b], Matthias Blüher[a,b,*]

[a]Department of Internal Medicine II, University of Cologne, Kerpener Str. 62, Koln 50924, Germany
[b]Department of Internal Medicine III, University of Leipzig, Leipzig, Germany

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Abstract

Caloric restriction and leanness have been shown to increase longevity in organisms ranging from yeast to mammals. Adipose tissue seems to be a pivotal organ in the aging process and in determination of lifespan. We have recently shown that fat-specific disruption of the insulin receptor gene is sufficient to increase lifespan in FIRKO mice, suggesting that reduced adiposity, even in the presence of normal or increased food intake, can extend lifespan. The model also suggests a special role for the insulin-signaling pathway in adipose tissue in the longevity process. Reduced fat mass has an impact on the duration of life in several other model organisms. In *Drosophila*, a specific reduction in the fat body through overexpression of forkhead type transcription factor (dFOXO) extends lifespan. Furthermore, sirtuin1 (SIRT1), the mammalian ortholog of the life-extending yeast gene silent information regulator 2 (*SIR2*), was proposed to be involved in the molecular mechanisms linking lifespan to adipose tissue. In the control of human aging and longevity, one of the striking physiological characteristics identified in centenarians is their greatly increased insulin sensitivity even compared with younger individuals. The effect of reduced adipose tissue mass on lifespan could be due to the prevention of obesity-related metabolic disorders including type 2 diabetes and atherosclerosis.

Keywords: Longevity; Aipose tissue; Insulin signaling; FIRKO; Animal models; SIRT1; dFOXO

1. Introduction

There are many theories of aging and parameters that influence lifespan, including genetic instability, telomerase activity and oxidative stress. Associated with the aging process is a progressive loss of physiological functions, both within individual cells and within the whole organism that increases the vulnerability to age-related health complications. The role of caloric restriction, metabolism, adipose tissue as well as insulin and insulin-like growth factor-1 (IGF-1) signaling in the aging process seems to be especially well conserved throughout evolution (Katic and Kahn, 2005). Systematic screening for longevity genes and the identification of several mutations that increase lifespan in diverse organisms including the yeast *Saccharomyces cerevisiae*, the nematode *Caenorhabditis elegans*, and the fruit fly *Drosophila melanogaster* support the concept that lifespan is genetically determined (Picard and Guarente, 2005). In diverse organisms and in all mammalian species studied so far, caloric restriction is the most potent environmental variable, which has been shown to extend lifespan (Weindruch and Walford, 1998). The effect of restricted feeding on lifespan has been studied in rodents for over 60 years, but it has been difficult to separate the beneficial effect of caloric restriction from that of reduced adipose tissue mass and leanness (Weindruch and Walford, 1998; Koubova and Guarente, 2003). The effects of caloric restriction on body fat and ageing have been widely studied (Das et al., 2004; Weindruch and Walford, 1998; Koubova and Guarente, 2003; Barzilai and Gabriely, 2001), however, this review does not primarily focus on experimental models investigating the consequences of caloric restriction.

Interestingly, calorie restricted obese mice had a greater maximum lifespan than ad-libitum fed normal mice, despite the fact, that calorie restricted obese mice had a higher body fat content, suggesting that caloric restriction does not primarily extent longevity by reducing adipose tissue mass (Harrison et al., 1984). If reduced adipose tissue mass leads

* Corresponding author. Address: Department of Internal Medicine II, University of Cologne, Kerpener Str. 62, Koln 50924, Germany. Tel.: +49 221 478 4176; fax: +49 221 478 3107.
E-mail addresses: matthias.blueher@uk-koeln.de, bluma@medizin.uni-leipzig.de (M. Blüher).

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to increased lifespan, one could speculate that ablation of white adipose tissue extends longevity. The consequences of transgenic lipoatrophy have been studied in several mouse models. However, most mouse models of lipoatrophy die shortly after birth or have at least a shortened lifespan (Ross et al., 1993; Moitra et al., 1998; Shimomura et al., 1998). These lipoatrophy models suggest that, despite the beneficial effects of reduced adipose tissue mass on extended lifespan, adipose tissue is required for a normal longevity most likely because of its role in maintaining whole body glucose homeostasis, lipid metabolism and insulin sensitivity.

What are potential molecular mechanisms linking the effect of caloric restriction on extended lifespan to the biology of adipose tissue? Caloric restriction produces a variety of biological effects, including reduction in adipose tissue, especially visceral adipose tissue (Barzilai et al., 1998), retardation of growth and development as well as a decrease in fertility (Holzenberger et al., 2004; Katic and Kahn, 2005; Das et al., 2004; Barzilai and Gabriely, 2001). Accumulation of adipose tissue is associated with an age-related decrease in insulin sensitivity, which subsequently leads to the development of obesity, diabetes, hypertension and atherosclerosis. In humans, these diseases strongly affect morbidity and mortality, especially among elderly (Reaven, 1988). Caloric restriction and reduced body fat mass blunt sexual maturation and fertility, which allows long-term survival through energy sparing and reverses age-related reduced insulin sensitivity (Picard and Guarente, 2005; Mora and Pessin, 2002). In addition, increased fat mass could contribute to the development of insulin resistance in the aging process through alterations in adipose-derived hormone production and increased free fatty acid release as a result of lipolysis in adipose tissue.

Studies over the last several years have revealed a central role of reduced fat mass and involvement of the insulin/IGF-1 pathway in the control of aging and longevity in diverse organisms from invertebrates to humans.

2. Insulin/IGF-1 signaling and longevity—from worms to humans

Although yeast do not have an insulin-signaling pathway, they appear to have precursors of such pathways that function in a glucose/nutrient-signaling cascade. There are strong similarities between these cascades in yeast with insulin and IGF-1 signaling systems in worms, flies, mammals and humans. These systems may be linked to metabolic regulation, oxidative stress resistance, food utilization, adipose tissue mass, and lifespan in each of these organisms. Such similarities suggest that the insulin/IGF-1 system is well evolutionary conserved and that it is a central component of an anti-aging system, which can be observed from yeast to humans (Katic and Kahn, 2005).

2.1. *Saccharomyces cerevisiae*

The yeast, *Saccharomyces cerevisiae*, is an attractive model to study the link between intracellular signaling pathways, metabolism and aging, because age-related alterations in metabolism and signaling can be analysed more easily in an unicellular eukaryote with a naturally short lifespan. Although yeast do not have an insulin-signaling pathway, they appear to have precursors of pathways that function in a glucose-signaling cascade. Screening for long-lived mutants in *S. cerevisiae* identified that mutations in genes, coding for precursors of such signaling molecules can extend the lifespan of non-dividing cells up to three-fold (Fabrizio et al., 2001).

2.2. *Caenorhabditis elegans*

Another powerful genetic model in the search for lifespan-controlling genes is the worm *Caenorhabditis elegans*, because of its small size, relatively short lifespan, rapid reproduction rate and well-characterized genetics. The insulin-signaling pathway consists of molecules encoded by the genes *daf-2* (the insulin/IGF-1 receptor homologue), *ins-7* (one out of at least 37 insulin-like ligands), *age-1* (similar to mammalian p110 catalytic subunit of PI 3-kinase), *akt-2* (homologue of Akt/PKB), *daf-16* (homologue of the forkhead family of transcription factors), and *daf-18* (PTEN homologue) (Katic and Kahn, 2005). Mutations of these genes led to extended lifespan by 50% or more and therefore revealed the importance of the insulin/IGF-1 signal transduction as a central regulator of longevity in *C. elegans* (Kimura et al., 1997; Kenyon et al., 1993). Interestingly, long-lived daf-2 mutants have increased fat accumulation in intestinal and hypodermal cells, suggesting that there is not a simply correlation between decreased fat mass and increased lifespan (Kimura et al., 1997).

2.3. *Drosophila melanogaster*—reduced fat body and longevity

The selection of *Drosophila melanogaster* lines, which display extended longevity, has been the major focus of genetics in experimental aging research. Fruit fly lines, which have been selected for extended longevity, are characterized by resistance to heat and desiccation, enhanced storage of lipid and glycogen, increased efficiency in the utilization of nutrients and by a greater metabolic capacity. Thus, the long-lived *Drosophila* lines share many of the phenotypes described in yeast and *C. elegans* with extended lifespan (Katic and Kahn, 2005). Mutations in genes homologue to the mammalian insulin/IGF-1 receptor-signaling pathway can increase life expectancy in *Drosophila* (Clancy et al., 2001; Tatar et al., 2001; Giannakou et al., 2004). It was shown, that mutation in the insulin receptor substrate homologue *chico* can extend lifespan in fruit flies (Clancy et al., 2001). Moreover, a mutation of

the *Drosophila* insulin-like receptor (*InR*) gene, which is homologous to mammalian insulin and IGF-1 receptors, significantly extends longevity in the flies (Tatar et al., 2001). Moreover, it was recently shown that activation of dFOXO in the *Drosophila* adult fat body, the fly equivalent of the mammalian liver and white adipose tissue, increased lifespan and reduced fecundity of female flies by 20–50%. No effect of dFOXO overexpression was reported in male flies (Giannakou et al., 2004). Expression of dFOXO, the homologue of mammalian FOXO, during early larval development causes inhibition of larval growth, alterations in feeding behaviour, and may lead to adults that are reduced in size due to a decreased cell size and cell number. These dFOXO-mediated alterations are similar to the effects of starvation in yeast and worms, suggesting a role for dFOXO in the response to nutritional changes. Taken together, these findings suggest that in *D. melanogaster* both reduction in adipose tissue mass and modification in the insulin-like signaling cascade play a significant role in the control of lifespan and aging.

2.4. Mice

Although decreased insulin-like signaling appears to increase life expectancy in invertebrates, whether the same is true in mammals or humans is unclear. At least three genes have been identified (*Pit1*^{dw}, *Prop1*^{df}, *Ghr*) in which naturally occuring loss-of-function mutations lead to dwarfism (*Prop-1*: Ames Dwarf mouse; *Pit-1*: Snell Dwarf mouse) with reduced levels of IGF-1 and insulin (Flurkey et al., 2001). These mice are deficient in serum growth hormone (GH), thyroid-stimulating hormone and prolactin, as well as IGF-1, which is normally secreted by the liver upon stimulation with GH and mediates most of its activity. Since mice that cannot release GH in response to GH-releasing hormone also live longer (Steger et al., 1993), it appeared that GH and IGF-1 deficiency mediated by the effects of mutations in *Prop-1* and *Pit-1* genes may be the most important determinants in the effect on longevity in the Dwarf mice (reviewed in: Katic and Kahn, 2005). Although IGF-1 knockout mice are not viable (Liu et al., 1993) and insulin receptor knockout mice die within one week after birth (Accili et al., 1996), a moderate decrease in IGF-1 receptor levels (Holzenberger et al., 2003) has been shown to extend longevity in mice. The loss of a single copy of *Igf1r* gene was shown to be sufficient to increase lifespan by 33% in females and 16% in males, accompanied by only a minimal reduction in growth (Holzenberger et al., 2003). In contrast to the naturally occuring long-lived Ames and Snell Dwarf mice, *Igf1r* heterozygous mice have no alteration in the age of sexual maturation and fertility. Serum IGF-1 concentrations are higher in *Igf1r* heterozygous mice compared to controls. Males tend to have higher fed glucose levels and impaired glucose tolerance, whereas the longer-lived females are more insulin sensitive and have lower fed plasma glucose concentrations (Holzenberger et al., 2003). Interestingly, *Igf1r* heterozygous mice are more resistant to oxidative stress, suggesting that increased lifespan of these mice could at least in part be due to resistance to oxidative stress (Holzenberger et al., 2003). Moreover, mice with a heterozygous *IGF-1R* knockout specifically in the central nervous system, which are genetically modified to be hyposensitive for IGF-1 in the brain, have an increased lifespan and a significant decrease in mortality (Holzenberger et al., 2004).

2.5. Rats

Using homozygous *dw/dw* rats with a specific and limited deficiency of GH and IGF-1, it was recently shown, that adult-onset growth hormone and IGF-1 deficiency reduces neoplastic disease, modifies age-related pathology, and increases lifespan (Sonntag et al., 2005). Moreover, moderate suppression of the GH-IGF-1 axis was shown to increase lifespan in a transgenic male Wistar rat model (Shimokawa et al., 2003). Caloric restriction in addition to the GH-IGF-1 suppression had an additive effect on extended lifespan, due to preventive effects of caloric restriction on selected diseases. This model suggests that caloric restriction affects aging and longevity by mechanisms other than suppression of the GH-IGF-1 axis, although CR might exhibit its effects partly through the reduced GH-IGF-1 axis (Shimokawa et al., 2003).

2.6. Humans

In humans, it was shown that polymorphisms in the IGF-1 receptor (*IGF-IR*) gene and genotype combinations in *IGF-IR* and *PI3KCB* genes, which are associated with low levels of free plasma IGF-1, are more frequent among long-lived people, suggesting that genetic variability at the insulin/IGF-I signaling response pathway may play a role in human longevity (Bonafe et al., 2003). On the other hand, severe, generalized loss-of-function mutations in insulin receptor function lead to severe insulin resistance and diabetes, which ultimately contributes to shortened lifespan in humans as well as in rodents (Katic and Kahn, 2005).

3. Extended longevity and adipose specific insulin resistance

Caloric restriction and reduced body fat mass were shown to increase lifespan, but it has been difficult to separate the effect of caloric restriction from that of leanness. Mice with fat-specific disruption of the insulin receptor gene (FIRKO) provide a model to investigate the effects of reduced adiposity on extended lifespan dissociated from the effects of caloric restriction, because FIRKO mice have a 50% reduced adipose tissue mass despite normal food intake (Blüher et al., 2002, 2003).

FIRKO mice are born with the expected frequency, survive well after weaning, are fertile and do not develop diabetes (Blüher et al., 2002). Growth curves were normal in male and female FIRKO mice from birth to 4 weeks of age, however, by 8 weeks of age, FIRKO mice had gained less weight than controls. Fasted and fed glucose concentrations are indistinguishable between FIRKO and control mice, whereas FIRKO mice have significantly lower fasted insulin concentrations compared to controls (Table 1). The most striking phenotype of FIRKO mice is their protection from age-related and hyperphagia-induced obesity and its associated insulin resistance and impaired glucose tolerance (Blüher et al., 2002). This phenotype leads to an extended longevity and demonstrates that reduced fat mass, even in the presence of normal or increased food intake, can extend lifespan (Blüher et al., 2003). Since reduced adiposity tends to result in lower insulin levels and protection from diabetes, the FIRKO mouse mimics at least some of the effects of calorie restriction without caloric restriction (Katic and Kahn, 2005). It has been demonstrated that caloric restriction reverses hepatic insulin resistance in aging rats by decreasing visceral fat mass (Barzilai et al., 1998). Therefore, reduced visceral fat mass in FIRKO mice could at least in part account for the phenotypic similarities between calorie-restricted animals and FIRKO mice. Moreover, adiponectin serum concentrations were significantly increased, especially in older FIRKO mice. Therefore, insulin sensitizing effects of elevated adiponectin levels in aging FIRKO mice might contribute to longevity in this animal model. The model also suggests a specific role for the insulin signaling pathway in adipose tissue in the longevity process. Little is known about the effect of other adipose tissue specific genetic alterations on longevity. Thus, no studies have been performed on the insulin receptor substrates, PI3-kinase or Akt with regard to lifespan (Katic and Kahn, 2005).

The glucose transporter 4 (GLUT4) is the major insulin-sensitive glucose transporter in white adipose tissue. It has been demonstrated that functional GLUT4 protein is essential for sustained growth, normal cellular glucose and fat metabolism and longevity. Whole body *GLUT4*$^{-/-}$ mice are characterized by growth retardation and decreased longevity associated with cardiac hypertrophy markedly reduced white adipose tissue mass accompanied by normoglycemia and a normal response to glucose load (Katz et al., 1995). However, selective inactivation of the *GLUT4* gene in adipose tissue had no effect on growth, body weight and fat mass in vivo, but these mice exhibit impaired insulin action in muscle and liver leading to glucose intolerance and insulin resistance (Abel et al., 2001). The effect of adipose-specific GLUT4 knockout on longevity has not been investigated.

Table 1
Comparison of phenotypic characteristics of a mouse model for extended longevity, the fat-specific insulin receptor knockout (FIRKO) mice with healthy human centenarians (Blüher et al., 2002; Paolisso et al., 1997)

Parameter	FIRKO mice (versus controls)	Centenarians (versus younger subjects)
Body mass index	↓	↓
Body fat content	↓	↓
WHR	NA	↓
Insulin sensitivity	↑	↑
Fasting plasma insulin	↓	↓
Plasma triglycerides	→	
Plasma LDL cholesterol	NA	↓
Plasma HDL cholesterol	NA	↑
Plasma FFA	→	↓
Plasma Leptin	↑	↑ (→)
Plasma IGF-1	→	↓

What are potential molecular mechanisms linking adipose tissue, the effect of food restriction and increased lifespan? Because overexpression of silent information regulator 2 (SIR2) ortholog, sirtuin 1 (SIRT1), the mammalian ortholog of the life-extending yeast gene SIR2, reduces adipogenesis and triglyceride accumulation in the lipid droplets of adipocytes, it was recently hypothesized that SIRT1 might be a candidate linking reduced adiposity to lifespan (Picard et al., 2004; Picard and Guarente, 2005). However, until now there is no experimental evidence that SIRT1 represents the molecular link between reduced adiposity and increased lifespan. In vivo, fasting and caloric restriction induce the recruitment of SIRT1 to PPARγ-response elements (PPREs) and promote lipolysis by inhibiting PPARγ-mediated fatty acid trapping. In addition, SIRT1 modulates the effects of PGC-1 α repression of glycolytic genes in response to fasting and pyruvate (Rodgers et al., 2005). Considering the insulin sensitizing effects of PPARγ agonists, it is at least controversial whether inhibition of PPARγ would be beneficial to human longevity. Moreover, it was recently shown that SIRT1 reduces p53-mediated apoptosis and that SIRT1 represses the activity of forkhead transcription factor FOXO3a and other mammalian forkhead factors. Therefore, it was speculated that downregulation of the damage-responsive p53 and FOXO3a may lead to reduced cancer incidence and favour a long lifespan under caloric restriction (Katic and Kahn, 2005). Finally, SIRT1 may link caloric restriction with forkhead-mediated metabolic changes including gluconeogenesis, insulin action, lipid usage and ketogenesis (Picard and Guarente, 2005). Thus, caloric restriction could extend lifespan by promoting the long-term survival of irreplaceable cells (Katic and Kahn, 2005).

4. Human longevity—the phenotype of a centenarian

In humans, the average lifespan in many developed countries is now more than 80 years, whereas only 200 years ago average lifespan was about 24 years due to high infant mortality, poor hygiene and the inability to treat infectious diseases (reviewed in: Katic and Kahn, 2005). In contrast,

maximum lifespan has not changed dramatically and seems to be stable at about 120 years (Fries, 1980). Thus, although the number of centenarians has increased, maximum human lifespan has not. There are many questions about biologic, genetic, evolutionary and social aspects of human longevity, but one of the most important questions is: What is the phenotype of a centenarian? One of the striking physiological characteristics of a centenarian is increased insulin sensitivity even compared with younger individuals (Paolisso et al., 1997, 2000). Healthy centenarians have in addition to more favourable anthropometric characteristics, including lower body fat content and increased insulin-mediated glucose uptake (Table 1), an increased plasma IGF-1/IGFBP-3 ratio compared with aged, but younger subjects. It was further shown that plasma IGF-I/IGFBP-3 ratio correlated with the BMI, body fat content, fasting plasma leptin, triglyceride, free fatty acid, and LDL cholesterol concentrations (Paolisso et al., 1997, Table 1). Moreover, data from 466 healthy individuals with an age range from 28 to 110 years demonstrated a significant reduction of insulin resistance in subjects from 90 to 100 years old, even after adjusting for body mass index (Paolisso et al., 2000). Although, there might be selection biases of a protected population or common longevity-favouring genetic or environmental factors in these studies, these data demonstrate that efficient insulin response, a non-atherogenic lipid profile and low body fat mass may have an impact on human longevity. Taken together, aging is associated with the progressive development of insulin resistance affecting insulin-mediated physiologic processes including glucose uptake in peripheral tissues, inhibition of lipolysis and hepatic control of glucose production (Picard and Guarente, 2005). However, the molecular mechanisms for age-related insulin resistance and fat accumulation are not completely understood.

5. Conclusions

Across different organisms, reduction in adipose tissue mass, either caused by caloric restriction, by naturally occuring mutations in insulin/IGF-1 signaling pathway or in genetically modified animals, increases lifespan. Studies of worms and fruit flies have revealed hundreds of genes and proteins that, when mutated, extend lifespan. Some of these genes encode components of insulin or insulin-like signaling pathways. Recent aging models suggest that insulin/ IGF receptors and signaling molecules could also play a role in the control of mammalian longevity. Extended lifespan in flies with overexpressed dFOXO in adult fat body and in mice with fat-specific disruption of the insulin receptor gene (FIRKO mice) demonstrate the important role of reduced adiposity and suggest a special role for the insulin signaling pathway in adipose tissue in the longevity process. However, distinct aspects of the physiologic role of insulin signaling in adipose tissue and their relationship to longevity still need to be elucidated.

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